Biochimica et Biophysica Acta 1817 (2012) S138–S141

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

S18 Supercomplexes / Proteomics

18L1

Identification of new assembly factors of complex I by complexome profiling

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Blue-native electrophoresis (BNE) is commonly used for the isolation and quantification of OXPHOS complexes. In order to study composition and dynamics of lower abundant mitochondrial complexes we combined BNE and label free quantitative mass spectrometry to analyze the complexome of isolated rat heart mitochondria. Hierarchical clustering of proteins with similar migration in BN-lanes allowed the identification of well-characterized mitochondrial complexes and unknown protein–protein interactions. The known complex I assembly factors CIA30, ECSIT and ACAD9 were found in a cluster together with TMEM126B, a protein of unknown function. Stable lentiviral knockdown of TMEM126B in the osteosarcoma cell line 143B resulted in a strong reduction of complex I levels without affecting the assembly of other OXPHOS complexes. Functional characterization by high-resolution respirometry of intact mitochondria from knockdown cells revealed a reduction of the complex I dependent respiration by roughly 65%. In contrast, succinate dependend respiration was unaffected. In addition, we characterized the homologous protein TMEM126A that was recently reported to be associated to mitochondrial diseases. Knockdown cells showed complex I deficiency but TMEM126A was not identified within the mitochondrial complex I assembly factor (MCIA) complex containing CIA30, ACAD9, ECSIT and TMEM126B. In summary, complexome profiling is a powerful bottom–up approach allowing identification of unknown protein interactions of an entire organelle.

doi:10.1016/j.bbabio.2012.06.363

18P1

Study of respiratory complexes in the low GC firmicutes *Bacillus subtilis* and *Geobacillus stearothermophilus*

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Rieske/cytochrome *b* complexes play a central role in energy production by translocating electrons and protons across the membrane, building up an electrochemical potential. This enzyme family is composed of several members, among them cytochrome bc_1 and b_6f complexes which have been shown to distinguish themselves with respect to the structure of their c-type cytochrome subunit and the co-factor content. Importantly an additional heme, heme c_i, is present in the quinone reduction side of b_6f complexes. Its function is not yet understood.

Here, we set out to study protein/protein and protein/lipid interfaces on the Rieske/cytochrome *b* complex from *Geaobacillus stearothermophilus*, a thermophilic low GC gram-positive bacterium. This enzyme is integrated in the respiratory chain of the bacterium and more closely related to cytochrome b_6f from photosynthetic reaction chains than to cytochrome bc_1 from the mitochondrial respiratory chain. At variance with mitochondrial bc_1 and chloroplasts b_6f complexes the enzyme of *G. stearothermophilus* uses menaquinone as a substrate. This complex has been enriched by anion exchange chromatography and density gradient centrifugation. Here we present a first characterization by UV/VIS and EPR spectroscopy.

In parallel, we started studying enzymes of the respiratory chain from *Bacillus subtilis*, a mesophilic bacterium, which is closely related to *G. stearothermophilus*. The respiratory complexes of these two organisms are quite similar, but exhibit different molecular organisations and stabilites.

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doi:10.1016/j.bbabio.2012.06.364

0005-2728/$ – see front matter.